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Failure of irradiated beef and ham to induce genetic aberrations in Drosophila

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1. Introduction

The use of ionizating radiation as a process to preserve food may soon be adopted much more widely if no harmful or unpleasant by-products are found to be induced by the irradiation. There have been conflicting reports regarding the production of mutagenic properties in food exposed to radiation and then fed to Drosophila. Negative results have been reported by Chopra (1965), Reddi, Reddy, Rao, Ebenezer and Rao (1965), Seecof and Kaplan (1966), and Khan and Alderson (1965) when irradiated food media or DNA was fed to Drosophila. An increase in the mutation rate in flies fed irradiated media was found by Swaminathan, Nirula, Natarajan and Sharm (1963), Rinehart and Ratty (1965, 1967), Holsten, Sugii and Steward (1965) and Parkash (1965).

Ham that had been irradiated by electrons and beef which had been exposed to gamma rays from ^{60}Co were fed to *Drosophila melanogaster* to determine whether meat sterilized by these methods would induce genetic aberrations. The loss of the X or Y chromosome and the induction of recessive sex-linked lethals were determined.

2. Materials and methods

The ham had been given an irradiation dose of 3·7–4·2 Mrad with electrons produced by the LINAC 10 MeV electron accelerator. The beef had been exposed to a 3 MCi ^{60}Co source for a dose of 4·7–7·1 Mrad by the U.S. Army Natick Research and Development Command at the Natick, Massachusetts facility. The radapportization (radiation process that sterilizes food) used for these meats was described by Heiligman, Wierbicki, Cohen and Mason (1976) and Wierbicki, Brynjolfsson, Johnson and Rowley (1975). Ten months later the radiation-sterilized ham or beef was finely ground to a syrup-like liquid by a Sorvall Omni-mixer. This was then added at a ratio of 2 g of processed meat to 2 g of Carolina 4–24 Drosophila medium plus 10 ml of water. Oregon R and yB/C(1)DX, yf/sc^{8}y^{+}Y stocks were reared on irradiated meat–Drosophila medium mixture. Adult males that emerged, having spent their entire larval life and had also fed as adults for 1 day on the test mixture, were used to determine the loss of the X and Y chromosome and induced sex-linked recessive lethals. The irradiated and non-irradiated meat–Drosophila media mixture caused a delay of several days in the period for the complete life cycle, i.e. it took 13–14 days to obtain adult flies from the egg stage reared at 24°C. The high-protein diet did slow down development. The controls consisted of beef that had been sterilized by thermal means, and beef that had been preserved by freezing. The third control

consisted of flies reared on the meatless Drosophila medium which was tested simultaneously with each of the four different meat-fed drosophila.

To determine whether feeding on the irradiated meat would induce the loss of the X and Y chromosomes and non-disjunction, 1-day old yB/sc^8y^+Y males which had been fed for their entire larval life on the test mixtures were mated to ywf females at a ratio of one male to three females. The males were transferred every 3 days to three different groups of females to obtain information on the various stages of spermatogenesis. The loss of the X or Y chromosome resulted in an exceptional ywf (yellow body, white eye, forked bristle) male, and if primary non-disjunction had occurred, exceptional $yB/ywf/sc^8y^+Y$, wild body, Bar eye, females would result.

The recessive sex-linked lethals were obtained by mating the 1-day-old Oregon R males that had spent their entire larval life on the various test mixtures to $In(1)sc^{8R}$, sc^{51} sc^8 w^a B(Muller-5) at a ratio of one male to three females for three 3-day-old broods. A recessive sex linked lethal was suspected if the F_1 female, when isolated, produced at least 8 Bar eye males and no wild type males. Five heterozygous F_2 females of each suspected lethal were isolated and the F_3 were examined, and if there were no wild type flies, a complete lethal was recorded.

3. Results

yB/sc^8y^+Y males which had fed on electron-irradiated ham, ^{60}Co-irradiated beef, thermally preserved beef, and frozen beef showed no significant increase in the loss of X or Y chromosomes or non-disjunction of these chromosomes. The data are presented in table 1. There was no significant increase in any of the broods.

Oregon R males which had spent their entire larval life on irradiated ham or beef, thermally preserved beef, and frozen beef, did not yield a significant increase in sex-linked recessive lethals. The data from these experiments are presented in table 2.

4. Discussion

The feeding of irradiated ham and beef to Drosophila males for their entire larval life did not induce significant increases in genetic aberrations. The analysis by a 2×2 contingency table of the recessive sex-linked lethals data does not provide evidence that irradiated ham or beef, when fed to Drosophila, will significantly increase lethals (see table 2). One could argue that the sampling size was too small (but with a total of 27 595 chromosomes tested for recessive lethals, this is not a strong argument), and that a 95 per cent confidence interval would not discriminate, if there was a difference due to the diet of the Drosophila. In our laboratory, the spontaneous mutation rate of Oregon R as used in various mutagenesis studies has averaged about 0·15 per cent, which is in line with the present data. The experiments involving the loss of the X chromosome cannot be deemed small, for the total numbers of flies examined was 162 435 and no significant increase in loss was induced by feeding irradiated beef and ham (see table 1). Although there have been reports from genetic studies that irradiated food has been mutagenic to Drosophila, attempts to reproduce these by some researchers failed. Swaminathan *et al.* (1963) who cultured Drosophila males food irradiated with 150 kR found an increase in recessive sex-linked and dominant lethals. Holsten *et al.* (1965) used 2 Mrad to irradiate sucrose and reported an increase in sex-linked recessive lethals in Drosophila males. Chopra (1965) irradiated DNA with 100 krad and yeast, sucrose and agar with 1 Mrad and found no increase in recessive lethals on the X and II chromosomes or in dominant lethals. Parkash (1965)

Brood (days)	Irradiated ham			Control			Irradiated beef			Control		
	Gametes	XO	XXY	Gametes	XO	XXY	Gametes	XO	XXY	Gametes	XO	XXY
0–3	13159	15	6	3963	5	3	23326	25	27	5106	6	5
3–6	9889	10	3	2003	3	1	18537	13	16	3250	3	4
6–9	5235	8	0	329	0	0	12091	9	5	1977	1	1
Totals	28282	33	9	6295	8	4	53954	47	48	10333	10	10
		(0·11%)	(0·032%)		(0·127%)	(0·063%)		(0·087%)	(0·089%)		(0·096%)	(0·096%)
		XO (0·08 to 0·165%)†, XXY (0·015 to 0·06%)†			XO (0·05 to 0·252%)†, XXY (0·017 to 0·16%)†			XO (0·002 to 0·12%)†, XXY (0·003 to 0·11%)†			XO and XXY (0·046 to 0·18%)	
		XO, $\chi^2=0·0002$‡ ($p=0·99$)			XXY, $\chi^2=0·664$‡ ($p=0·42$)			XO, $\chi^2=0·015$‡ ($p=0·90$)			XXY, $\chi^2=(0·004$‡ ($p=0·95$)	

Brood (days)	Thermally preserved beef			Control			Frozen beef			Control		
	Gametes	XO	XXY	Gametes	XO	XXY	Gametes	XO	XXY	Gametes	XO	XXY
0–3	11085	14	6	3272	2	0	12822	17	12	2491	5	4
3–6	8397	4	12	2036	1	3	15246	16	11	2991	6	2
6–9	3790	2	1	1153	0	1						
Totals	23272	20	19	6461	3	4	28068	33	23	5482	11	6
		(0·086%)	(0·082%)		(0·046%)	(0·062%)		(0·118%)	(0·082%)		(0·20%)	(0·11%)
		XO (0·05 to 0·13%)†, XXY (0·04 to 0·12)†			XO (0·0096 to 0·14%)†, XXY (0·017 to 0·168%)†			XO (0·08 to 0·166%)†, XXY (0·05 to 0·12%)†			XO (0·10 to 0·36%)†, XXY (0·04 to 0·24%)	
		XO, $\chi^2=0·574$‡ ($p=0·45$)			XXY, $\chi^2=0·063$‡ ($p=0·45$)			XO, $\chi^2=1·824$‡ ($p=0·18$)			XXY, $\chi^2=0·146$‡ ($p=0·70$)	

† 95 per cent binomial confidence interval (Bliss 1967).

‡ 2 × 2 contingency table with Yates correction between control and treated meat.

Table 1. The incidence of the loss of X and Y chromosomes and non-disjunction in gametes of yB/sc^8y^+ males reared on a 50 per cent mixture of irradiated meat and Drosophila medium. The controls are males reared only on Drosophila medium.

Brood (days)	Irradiated ham: Number of chromosomes	Irradiated ham: Recessive lethals	Control: Number of chromosomes	Control: Recessive lethals	Irradiated beef: Number of chromosomes	Irradiated beef: Recessive lethals	Control: Number of chromosomes	Control: Recessive lethals
0–3	1813	4	1768	2	2963	3	721	1
3–6	1312	0	694	0	1787	1	370	0
6–9	671	1			2341	1	366	0
Totals	3796	5 (0·13%) (0·04 to 0·31%)† $\chi^2=0{\cdot}04$‡ ($p=0{\cdot}60$)	2462	2 (0·081%) (0·001 to 0·293)†	7091	5 (0·071%) (0·023 to 0·16%)† $\chi^2=0{\cdot}27$‡ ($p=0{\cdot}60$)	1457	1 (0·069%) (0·0017 to 0·38%)†

Brood (days)	Thermally preserved beef: Number of chromosomes	Thermally preserved beef: Recessive lethals	Control: Number of chromosomes	Control: Recessive lethals	Frozen beef: Number of chromosomes	Frozen beef: Recessive lethals	Control: Number of chromosomes	Control: Recessive lethals
0–3	2779	3	898	1	2166	4	611	4
3–6	1632	0	650	0	2033	1	214	1
6–9	233	0	93	0	1135	0	318	0
Totals	4644	3 (0·065%) (0·013 to 0·19%)† $\chi^2=0{\cdot}27$‡ ($p=0{\cdot}60$)	1641	1 (0·061%) (0·0015 to 0·293%)†	5334	5 (0·19%) (0·046 to 0·21%)† $\chi^2=5{\cdot}23$‡ ($p=0{\cdot}02$)	1143	5 (0·44%) (0·142 to 1·01%)†

† 95 per cent bionomial confidence interval (Bliss 1967).
‡ 2 × 2 contingency table with Yates correction factor between control and treated meat.

Table 2. The incidence of sex-linked recessive lethals from Oregon R males reared on a 50 per cent mixture of irradiated meat and Drosophila medium. The controls are males reared only on Drosophila medium.

had reported earlier that DNA irradiated with 100 kR induced recessive lethals in Drosophila. However, Khan and Alderson (1965) also used 100 kR to irradiate DNA and found no differences between unirradiated DNA in the production of recessive lethals in Drosophila. Seecof and Kaplan (1966) also failed to induce lethals in Drosophila fed DNA irradiated with 100 kR of X-rays. Reddi *et al.* (1965) irradiated Drosophila medium with 150 and 300 kR and fed it to Drosophila, but it did not induce recessive lethals. Rinehart and Ratty (1965, 1967) irradiated Drosophila media with 150, 500, and 3000 kR which when fed to males caused a small and consistent increase in recessive lethals. Irradiated food aged 3 weeks before use still produced a greater, but non-significant increase in lethals. Sucrose was irradiated with 300 kR by a linear accelerator and was found to be non-mutagenic in Drosophila. The slight increase in lethals as a result of feeding irradiated media was claimed to be due to an increase in gonial mutations.

In the work reported here, ham was irradiated by electrons to a dose of 3·7–5·2 Mrad and beef was exposed to 4·7–7·1 Mrad of gamma rays. These were larger than any dose of radiation reported in the literature in the mutagenic studies of feeding irradiated food to Drosophila. No significant increases were induced in recessive sex-linked lethals, loss of chromosomes or non-disjunction. If radiation did induce mutagenic substances in food, an increase in radiation should then have produced a proportional increase in genetic aberrations. Could the 10-month waiting period before use have eliminated the mutagenic substances? There is no doubt that irradiation processes could have produced peroxides and free radicals, for the meat products are about 70 per cent water. These are extremely reactive substances and relatively short lived. Heiligman *et al.* (1976) reported that there was no initial difference in the peroxide levels, which were low, in frozen or thermally preserved beef, compared to electron- or gamma-irradiated beef. Storage had no effect on peroxides in any of these groups. The irradiation produced no stable mutagenic compounds. Since the males had fed on the irradiated food, both for their entire larval life and also as adults the entire spectrum of spermatogenesis was thus sampled by the brooding technique. There was no induced significant increase in the genetic aberrations tested in any of the cells in spermatogenesis.

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